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TITLE: LIMITED TOXICITY AND MUTAGENICITY TESTING OF FIVE UNICHARGE PROPELLANT COMPOUNDS

SUBTITLE: Evaluation of Five Unicharge Propellants in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

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N-methyl-,n-ethyl- and n-butyl-2nitratoethyl nitramine (MeNENA, EtNENA and BuNENA) and bis (2,2-dinitropropyl) acetal/formal (~50/50 mixture) ± diphenyl amine stabilizer (BDNPA/F±DPA) were tested for delayed contact hypersensitivity in the Buehler Assay. Three groups of guinea pigs per study received the test article, positive or negative control by topical application once per week for a total of three six-hour insult periods. Fourteen days after the last induction all animals were challenged at a naive site. Desired responses were elicited in the negative and positive control groups. No responses were observed after challenge in any test animal. Based upon the results of these assays, MeNENA, EtNENA, BuNENA, BDNPA/F±DPA did not cause dermal sensitization in guinea pigs.

14. SUBJECT TERMS

Beuhler Assay, Unicharge, Propellants, Methyl-NENA, Ethyl-NENA, butyl-NENA, bis-(2,2-dinitropropyl)acetal, bis-(2,2 dinitropropyl) acetal, bis-(2,2-dinitropropyl) formal, lab animals, RAIII

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Evaluation of Five Unicharge Propellants in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

EXECUTIVE SUMMARY

In preliminary dose-range-finding studies to determine the irritation potential of five unicharge propellants, two male and two female guinea pigs per study were each exposed to three concentrations of 10, 20 and 50% of the test material in acetone and as received. Based upon the results of the dose-range-finding studies, the test articles were dosed as received.

The potential of each test article to cause dermal sensitization in guinea pigs was determined by repetitive dermal application. Three groups of guinea pigs per study received the test article, 0.3% 1-chloro-2,4-dinitrobenzene (positive control), or distilled water or 80% ethanol (negative control), respectively, by topical application once per week for a total of three six-hour insult periods. No signs of erythema were observed in the test article-treated or negative control animals during the induction phase of the study. No to severe erythema with/without edema was observed in the positive control animals during the induction phase of the study. Fourteen days after the last insult, all animals were challenged at a previously untested site. A positive response was elicited in the animals treated with the positive control article. No responses were observed in any test article-treated animal. No responses were observed in any control animal challenged with the vehicle on the left flank and test article on the right flank. One bis (2,2-dinitropropyl) acetal/formal (~50/50 mixture) + diphenyl amine stabilizer treated animal was euthanized during the study due to a prolapsed rectum. Necropsy of this animal revealed no other visible lesions. This occurence was considered spurious in nature and not related to test article administration. All other animals gained weight and survived to study termination.

Based upon the observations made in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay), test articles n-methyl-2 nitratoethyl nitramine, n-ethyl-2 nitratoethyl nitramine, n-butyl-2 nitratoethyl nitramine, bis (2,2-dinitropropyl) acetal/formal (~50/50 mixture) + diphenyl amine stabilizer and bis (2,2-dintropropyl) acetal/formal (~50/50 mixture)-diphenyl amine stabilizer did not cause dermal sensitization in guinea pigs under the conditions of this study. Dermal sensitization was elicited in the animals receiving the positive control article, 1-chloro-2,4-dinitrobenzene at a 0.3% concentration.

Evaluation of Five Unicharge Propellants in the Delayed Contact Hypersensitivity in Guinea Pigs (Buehler Assay)

PH 424-US-001...005-91

STUDY DESCRIPTION

Sponsor:	U.S. Army Medical Research and Development Laboratory Fort Detrick Frederick, MD 21702-5010
Testing Facility:	Pharmakon Research International, Inc. P.O. Box 609 Waverly, PA 18471
Test Facility Study Conduct S.O.P. No.:	PH-424
Study Numbers:	PH 424-US-001-91 PH 424-US-002-91 PH 424-US-003-91 PH 424-US-004-91 PH 424-US-005-91
Purpose of the Study:	To determine the potential of a product to promote skin sensitization after repeated topical "skin insult" applications.
Ownership of the Study:	The sponsor owns the study. All raw data, analysis, and reports are the property of the sponsor.
Study Monitor:	Major Nathaniel Powell, U.S. Army Medical Research and Development Laboratory
Study Director:	Susan E. Armondi, LAT, Pharmakon Research International, Inc.
Technical Performance:	Susan E. Armondi, LAT, Charles Boyarsky, LAT, John Morahan, B.S., LAT, Kim DiLeo, B.S., LAT, Maura J. Bieszczad, Shirley Chappuis, A.S., AVT, LAT and Christine L. Hocke, B.A., LAT
Q.A.U. Responsible Personnel:	Leslie J. Pinnell, M.S.
Date Study Director Signed Protocols:	September 23, 1991

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Dates of Technical Performance:	Dose-Range-Finding Studies: October 12, 1991 through October 13, 1991 and October 29, 1991 through October 30, 1991. Assays: November 7, 1991 through December 7, 1991 and November 8, 1991 through December 8, 1991
Good Laboratory Practices Statement:	These studies were conducted in compliance with the Good Laboratory Practice Regulations except that stability samples for archive retention were not maintained at the testing facility due to the nature of the compounds. Stability samples were returned to the sponsor and the retention of the samples will be the responsibility of the sponsor. There were no other deviations from the GLP Regulations which affected the quality or integrity of the study. Q.A.U. findings from the inspections conducted of this study and from the audit of the final report are documented and have been provided to the study director and the test facility management.
Records Maintained:	All raw data, final report documentation and protocol will be maintained in the archives of Pharmakon Research International, Inc.
Recordings:	Standard Pharmakon Notebook
Notebook Reference:	Notebook #1532, pages 246-296

TEST ARTICLES

TEST ARTICLE	DESCRIPTION	LOT #	CAS #	DATE(S) SUBMITTED
n-methyl-2-nitratoethyl nitramine (MeNENA)	white solid	XAP-MeNENA	17096-47-8	9/19/91
n-ethyl-2-nitratoethyl nitramine (EtNENA)	yellow liquid	XAP-EtNENA-4B	85068-73-1	9/19/91, 11/29/91
n-butyl-2-nitratoethyl nitramine (BuNENA)	yellow liquid	XAP-BuNENA-15B	82486-82-6	9/19/91

TEST ARTICLE	DESCRIPTION	LOT #	CAS #	DATE(S) SUBMITTED
bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer (BDNPA/F+DPA)	yellow liquid	Set #1	5108-69-0	9/19/91, 12/5/91
bis-(2,2-dinitropropyl) formal without dipenyl amine stabilizer (BDNPA/F-DPA)	yellow liquid	Set #2	5917-61-3	9/19/91, 12/5/91

Analysis of Purity: The purity, identity, strength and stability of the test articles were the responsibility of the sponsor. Stability samples for archive retention were not maintained at the testing facility due to the nature of the compounds. Samples were returned to the sponsor and the retention of the samples will be the responsibility of the sponsor.

POSITIVE CONTROL ARTICLE

Positive Control Article: 1-Chloro-2,4-dinitrobenzene (DNCB)

Supplier (Source): Aldrich Chemical Company, Milwaukee, Wisconsin

Lot No.: 01204PP

Description: Yellow crystal

How Supplied: 50 gram bottle

Dose Concentration: 0.3%

Special Handling Instructions: Standard precautions

Purity: 99+%

Analysis of Purity:	The purity of the positive control article was determined by the manufacturer. A certificate of analysis is maintained in the archives of Pharmakon Research, International, Inc.
Rationale for Positive Control Article:	DNCB is a recognized sensitization agent with guinea pigs.

TEST SYSTEM

Species:	Guinea Pig
Strain:	Hartley
Supplier (Source):	BuckberG Lab Animals, Tomkins Cove, New York
Sex:	Male and female
Age at Initiation:	Generally 4 to 6 weeks
Protocol Weight Range:	300-700 grams
No. on Study:	Four (4) per Dose-Range-Finding Study Thirty-five (35) per Assay
Method and Justification for Randomization:	Selection based upon body weight, sex and apparent good health.
Acclimation Period:	Minimum of five (5) days
System of Identification:	Cages marked with animal number, dose level and sex. Guinea pigs were ear tagged.

HUSBANDRY

Research Facility Registration:	U.S.D.A. Registration No. 23-R-107 under the Animal Welfare Act 74: SC 2131 et seq.
Animal Rooms:	Separate isolation by test system Light cycle - 12 hours light, 12 hours dark Temperature/Relative Humidity - Every attempt was made to maintain a temperature of 22°C ± 3°C (66-77°F) and a relative humidity of 30 to 70%.

Any excursions outside the temperature or humidity ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.

Housing: Guinea pigs were housed individually in ½" stainless steel wire mesh cages sized in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.

Sanitization: Waste material was removed daily. Cages and feeders sanitized every two weeks.

Food: Purina Guinea Pig Diet^{R}, ad libitum, food was checked daily and added or replaced as needed. Feeders are designed to reduce soiling, bridging and scattering.

Food Analysis: There were no contaminants that were reasonably expected to be present in the dietary material known to be capable of interfering with the purpose or conduct of the study.

Water: Fresh tap water, ad libitum.

Water Analysis: Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

METHODS

Rationale for Test System: This test will readily detect moderate to strong skin sensitizers and thus act as an early warning against the use of such materials for human testing.

Compound Preparation: Dose-Range-Finding Studies - 1.0, 10 and 50% suspensions in acetone and dosed as received from sponsor.

Definitive Studies - MeNENA dosed as received after dessication. All other test articles were dosed as received.

Dose Administration: MeNENA - 300 mg/site and moistened with 0.3 ml distilled water. All other test articles were administered at a volume of 0.3 ml/site.

Rationale for Dose Selection: Determine by pre-screening various dose levels on naive animals (Dose-Range-Finding Studies).

Evaluation of Five Unicharge Propellants in the Delayed Contact Hypersensitivity in Guinea Pigs (Buehler Assay)
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Route of Administration:	Dermal
Rationale for Route of Administration:	Recommended route for human use
Frequency of Administration:	Three (3) six hour inductions: test article as received (experimental animals) Three (3) six hour inductions: DNCB (0.3%) (positive control animals) Three (3) six hour inductions: Distilled water or 80% ethanol (negative control animals) One (1) six hour challenge: test article as received (experimental animals) One (1) six hour challenge: DNCB (0.3%) (positive control animals) One (1) six hour challenge: test article (right flank), vehicle (left flank) (Negative Control animals)
No. of Animals Per Dose Group, Per Compound:	Dose-range - four (4) Test article - twenty (20) Positive control - five (5) Negative control - ten (10)
Length of Studies:	Dose-Range-Finding Study - Twenty-four (24) hours Definitive Study - Thirty (30) days
Dose-Range-Finding Studies:	Prior to initiation of the studies, the irritation potential of each test article was determined in a dose-range-finding study. Four (4) previously unexposed animals were each exposed to four concentrations of 1.0, 10 and 50% of the test article in acetone and as received, by the technique described in site preparation. The procedure described in Table I (page 12) for primary challenge was used in grading the responses except that only 24 hour grades were obtained. No signs of erythema were observed at any treatment site with the exception of slightly patchy erythema in one animal treated with EtNENA as received. Therefore, the decision was made to dose the test articles in the definitive studies as received.
Site Preparation:	The left dorsal surface of each animal was clipped free of hair approximately twenty-four hours prior to each application of the test material. The test material was applied to twenty guinea pigs. A concurrent positive

control group consisting of five guinea pigs was treated with (0.3%) 1-chloro-2,4-dinitrobenzene in ethanol (80%). An additional group of twenty guinea pigs was treated with the negative control article (distilled water or 80% ethanol).

Induction Sensitization Exposure:

All materials were applied beneath a 25 mm Hill Top Chamber®, (Hill Top Research, Inc., Miamiville, Ohio) and covered with dental dam. The patch and dam were held in place with clips attached to the sides of the guinea pig restrainer. The patches were allowed to remain in place for six hours, after which the rubber dams and patches were removed. This procedure was performed once per week for three weeks, a total of three six hour inductions. The treated sites were examined after each dosing day and scored at 24 and 48 hours for erythema according to Table I.

Challenge:

Fourteen days after the last induction exposure, all animals were challenged in the same manner on a naive site on the left flank. The negative control animals were challenged with distilled water or 80% ethanol on the left flank and test article on the right flank. Twenty-four hours after challenge, all animals were depilated with Neet® Hair Remover (Whitehall Laboratories, Inc., New York 10017). The depilatory was applied to the test sites and surrounding areas and removed within 30 minutes by thoroughly rinsing with water and gently patting dry. They were then returned to their cages. A minimum of two hours after depilation, test sites were graded for erythema according to Table I. The scoring was repeated twenty-four hours later (48 hour grade).

Interpretation of Results:

All test sites were graded on a scale of 0 to 3 as follows:

Table I

Evaluation of Dermal Irritation (Erythema)
0 = No erythema
+ = Slightly patchy erythema
1 = Slight or confluent or moderate patchy erythema or area
2 = Moderate erythema
3 = Severe erythema with/without edema

Grades of 1 or greater for erythema in the test group indicate sensitization, provided such grades are not seen on negative control animals challenged with the test article. The reactions of test animals that exceed the most severe control reaction are presumed to be due to sensitization. Incidence is the number of positive animals in each group divided by the total number of animals tested in that group. Severity is calculated as the sum of the test grades divided by the total number of animals tested in a given group determined for 24 and 48 hours. All average grades are rounded off to the nearest tenth of a unit. Individual scores during induction and challenge may be found in Tables II through VI. Incidence and severity scores are found in Table VII. Body weight data are presented in Tables VIII through XI.

RESULTS

One BDNPAF/+DPA-treated animal was euthanized during the study due to a prolapsed rectum. Necropsy of this animal revealed no other visible lesions. This occurrence was considered spurious in nature and not related to the test article administration. All other animals gained weight and survived to study termination. No signs of erythema were observed in the test article treated or negative control animals during the induction phase of the study. No to severe erythema with/without edema was observed in the positive control animals during the induction phase of the study. Fourteen days after the last insult, all animals were challenged at a previously untested site. A positive response was elicited in the animals treated with the positive control article. No responses were observed in any test article-treated animals. No response were observed in any negative control animal challenged with vehicle on the left flank and test article on the right flank.

CONCLUSION

Based upon the observations made in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay), test articles MeNENA, EtNENA, BuNENA, BDNPA/F+DPA and BDNPA/F-DPA did not cause dermal sensitization in guinea pigs under the conditions of this study. Dermal

sensitization was elicited in the animals receiving the positive control article, 1-chloro-2,4-dinitrobenzene at a 0.3% concentration.

REFERENCE

Ritz, H. L. and E. V. Buehler, 1980. Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests. Current Concepts in Cutaneous Toxicity, ed. Drill, V. A. and T. Lazar. Academic Press, Inc., pp. 25-40.

Table II. Evaluation of MeNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Individual Animal Scores

PH 424-US-001-91

"Treatment Group"	Animal No.	Sex	Induction Week 1 Reading (hrs) 24	48	Induction Week 2 24	48	Induction Week 3 24	48	Challenge Week 5 24	48
"Test Group" (MeNENA)	3801	M	0	0	0	0	0	0	0	0
	3802	M	0	0	0	0	0	0	0	0
	3803	M	0	0	0	0	0	0	0	0
	3804	M	0	0	0	0	0	0	0	0
	3805	M	0	0	0	0	0	0	0	0
	3806	M	0	0	0	0	0	0	0	0
	3807	M	0	0	0	0	0	0	0	0
	3808	M	0	0	0	0	0	0	0	0
	3809	M	0	0	0	0	0	0	0	0
	3810	M	0	0	0	0	0	0	0	0
	3811	F	0	0	0	0	0	0	0	0
	3812	F	0	0	0	0	0	0	0	0
	3813	F	0	0	0	0	0	0	0	0
	3814	F	0	0	0	0	0	0	0	0
	3815	F	0	0	0	0	0	0	0	0
	3816	F	0	0	0	0	0	0	0	0
	3817	F	0	0	0	0	0	0	0	0
	3818	F	0	0	0	0	0	0	0	0
	3819	F	0	0	0	0	0	0	0	0
	3820	F	0	0	0	0	0	0	0	0
"Positive Control" (0.3% DNCB)	3831	M	0	0	0	0	1	2	3	2
	3832	M	0	0	+	+	2	3	2	1
	3833	M	1	1	+	0	1	2	3	2
	3834	F	1	1	+	+	2	1	2	2
	3835	F	0	0	+	+	3	3	3	3
									L R	L R
"Negative Control" (distilled water)	3821	M	0	0	0	0	0	0	0 0	0 0
	3822	M	0	0	0	0	0	0	0 0	0 0
	3823	M	0	0	0	0	0	0	0 0	0 0
	3824	M	0	0	0	0	0	0	0 0	0 0
	3825	M	0	0	0	0	0	0	0 0	0 0
	3826	F	0	0	0	0	0	0	0 0	0 0
	3827	F	0	0	0	0	0	0	0 0	0 0
	3828	F	0	0	0	0	0	0	0 0	0 0
	3829	F	0	0	0	0	0	0	0 0	0 0
	3830	F	0	0	0	0	0	0	0 0	0 0

L = Left flank (distilled water)
R = Right flank (MeNENA)
ACT/MAS(ARMY)

Table III. Evaluation of EtNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Individual Animal Scores

PH 424-US-002-91

"Treatment Group"	Animal No.	Sex	Induction Week 1 Reading (hrs) 24	48	Induction Week 2 24	48	Induction Week 3 24	48	Challenge Week 5 24	48
"Test Group" (EtNENA)	3836	M	0	0	0	0	0	0	0	0
	3837	M	0	0	0	0	0	0	0	0
	3838	M	0	0	0	0	0	0	0	0
	3839	M	0	0	0	0	0	0	0	0
	3840	M	0	0	0	0	0	0	0	0
	3841	M	0	0	0	0	0	0	0	0
	3842	M	0	0	0	0	0	0	0	0
	3843	M	0	0	0	0	0	0	0	0
	3844	M	0	0	0	0	0	0	0	0
	3845	M	0	0	0	0	0	0	0	0
	3846	F	0	0	0	0	0	0	0	0
	3847	F	0	0	0	0	0	0	0	0
	3848	F	0	0	0	0	0	0	0	0
	3849	F	0	0	0	0	0	0	0	0
	3850	F	0	0	0	0	0	0	0	0
	3851	F	0	0	0	0	0	0	0	0
	3852	F	0	0	0	0	0	0	0	0
	3853	F	0	0	0	0	0	0	0	0
	3854	F	0	0	0	0	0	0	0	0
	3855	F	0	0	0	0	0	0	0	0
"Positive Control" (0.3% DNCB)	3866	M	0	0	+	+	1	2	3	2
	3867	M	0	0	+	+	1	3	3	3
	3868	F	0	0	+	+	1	1	3	2
	3869	F	0	0	1	1	+	+	3	3
	3870	F	1	1	1	1	3	3	3	3
									L R	L R
"Negative Control" (80% ethanol)	3856	M	0	0	0	0	0	0	0 0	0 0
	3857	M	0	0	0	0	0	0	0 0	0 0
	3858	M	0	0	0	0	0	0	0 0	0 0
	3859	M	0	0	0	0	0	0	0 0	0 0
	3860	M	0	0	0	0	0	0	0 0	0 0
	3861	F	0	0	0	0	0	0	0 0	0 0
	3862	F	0	0	0	0	0	0	0 0	0 0
	3863	F	0	0	0	0	0	0	0 0	0 0
	3864	F	0	0	0	0	0	0	0 0	0 0
	3865	F	0	0	0	0	0	0	0 0	0 0

L = Left flank (80% ethanol)
R = Right flank (EtNENA)

Table IV. Evaluation of BuNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Individual Animal Scores

PH 424-US-003-91

"Treatment Group"	Animal No.	Sex	Induction Week 1 Reading (hrs) 24	48	Induction Week 2 24	48	Induction Week 3 24	48	Challenge Week 5 24	48
"Test Group" (BuNENA)	3871	M	0	0	0	0	0	0	0	0
	3872	M	0	0	0	0	0	0	0	0
	3873	M	0	0	0	0	0	0	0	0
	3874	M	0	0	0	0	0	0	0	0
	3875	0	0	0	0	0	0	0	0	
	3876	M	0	0	0	0	0	0	0	0
	3877	M	0	0	0	0	0	0	0	0
	3878	M	0	0	0	0	0	0	0	0
	3879	M	0	0	0	0	0	0	0	0
	3880	M	0	0	0	0	0	0	0	0
	3881	F	0	0	0	0	0	0	0	0
	3882	F	0	0	0	0	0	0	0	0
	3883	F	0	0	0	0	0	0	0	0
	3884	F	0	0	0	0	0	0	0	0
	3885	F	0	0	0	0	0	0	0	0
	3886	F	0	0	0	0	0	0	0	0
	3887	F	0	0	0	0	0	0	0	0
	3888	F	0	0	0	0	0	0	0	0
	3889	F	0	0	0	0	0	0	0	0
	3890	F	0	0	0	0	0	0	0	0
"Positive Control" (0.3% DNCB)	3901	M	1	1	+	+	2	3	3	3
	3902	M	1	1	1	1	2	2	3	3
	3903	M	0	1	+	+	2	1	3	3
	3904	F	0	0	+	+	1	1	2	2
	3905	F	0	0	+	+	1	1	3	3
									L R	L R
"Negative Control" (80% ethanol)	3891	M	0	0	0	0	0	0	0 0	0 0
	3892	M	0	0	0	0	0	0	0 0	0 0
	3893	M	0	0	0	0	0	0	0 0	0 0
	3894	M	0	0	0	0	0	0	0 0	0 0
	3895	M	0	0	0	0	0	0	0 0	0 0
	3896	F	0	0	0	0	0	0	0 0	0 0
	3897	F	0	0	0	0	0	0	0 0	0 0
	3898	F	0	0	0	0	0	0	0 0	0 0
	3899	F	0	0	0	0	0	0	0 0	0 0
	3900	F	0	0	0	0	0	0	0 0	0 0

L = Left flank (80% ethanol)
R = Right flank (BuNENA)

Table V. Evaluation of BDNPA/F+DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Individual Animal Scores

PH 424-US-004-91

"Treatment Group"	Animal No.	Sex	Induction Week 1 Reading (hrs) 24	48	Induction Week 2 24	48	Induction Week 3 24	48	Challenge Week 5 24	48
"Test Group" (BDNPA/F +DPA)	3906	M	0	0	0	0	0	0	0	0
	3907	M	0	0	0	0	0	0	0	0
	3908	M	0	0	0	0	0	0	0	0
	3909	M	0	0	0	0	0	0	0	0
	3910	0	0	0	0	0	0	0	0	0
	3911	M	0	0	e	-	-	-	-	-
	3912	M	0	0	0	0	0	0	0	0
	3913	M	0	0	0	0	0	0	0	0
	3914	M	0	0	0	0	0	0	0	0
	3915	M	0	0	0	0	0	0	0	0
	3916	F	0	0	0	0	0	0	0	0
	3917	F	0	0	0	0	0	0	0	0
	3918	F	0	0	0	0	0	0	0	0
	3919	F	0	0	0	0	0	0	0	0
	3920	F	0	0	0	0	0	0	0	0
	3921	F	0	0	0	0	0	0	0	0
	3922	F	0	0	0	0	0	0	0	0
	3923	F	0	0	0	0	0	0	0	0
	3924	F	0	0	0	0	0	0	0	0
	3925	F	0	0	0	0	0	0	0	0
"Positive Control" (0.3% DNCB)	3936	M	1	+	+	+	2	2	3	3
	3937	M	1	0	+	+	1	0	2	3
	3938	F	0	0	1	1	3	3	3	3
	3939	F	0	+	+	1	3	3	2	2
	3940	F	0	0	+	1	1	2	3	3
									L R	L R
"Negative Control" (80% ethanol)	3926	M	0	0	0	0	0	0	0 0	0 0
	3927	M	0	0	0	0	0	0	0 0	0 0
	3928	M	0	0	0	0	0	0	0 0	0 0
	3929	M	0	0	0	0	0	0	0 0	0 0
	3930	M	0	0	0	0	0	0	0 0	0 0
	3931	F	0	0	0	0	0	0	0 0	0 0
	3932	F	0	0	0	0	0	0	0 0	0 0
	3933	F	0	0	0	0	0	0	0 0	0 0
	3934	F	0	0	0	0	0	0	0 0	0 0
	3935	F	0	0	0	0	0	0	0 0	0 0

L = Left flank (80% Ethanol)
R = Right flank (BDNPA/F+DPA)
e = animal euthanized
- Not Applicable

Table VI. Evaluation of BDNPA/F-DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Individual Animal Scores

PH 424-US-005-91

"Treatment Group"	Animal No.	Sex	Induction Week 1 Reading (hrs) 24	1 48	2 24	2 48	3 24	3 48	Challenge 5 24	5 48
"Test Group"										
(BDNPA/F-DPA)	3941	M	0	0	0	0	0	0	0	0
	3942	M	0	0	0	0	0	0	0	0
	3943	M	0	0	0	0	0	0	0	0
	3944	M	0	0	0	0	0	0	0	0
	3945	M	0	0	0	0	0	0	0	0
	3946	M	0	0	0	0	0	0	0	0
	3947	M	0	0	0	0	0	0	0	0
	3948	M	0	0	0	0	0	0	0	0
	3949	M	0	0	0	0	0	0	0	0
	3950	M	0	0	0	0	0	0	0	0
	3951	F	0	0	0	0	0	0	0	0
	3952	F	0	0	0	0	0	0	0	0
	3953	F	0	0	0	0	0	0	0	0
	3954	F	0	0	0	0	0	0	0	0
	3955	F	0	0	0	0	0	0	0	0
	3956	F	0	0	0	0	0	0	0	0
	3957	F	0	0	0	0	0	0	0	0
	3958	F	0	0	0	0	0	0	0	0
	3959	F	0	0	0	0	0	0	0	0
	3960	F	0	0	0	0	0	0	0	0
"Positive Control"	3971	M	1	+	+	+	3	3	3	3
(0.3% DNCB)	3972	M	1	+	+	+	1	+	2	1
	3973	F	0	+	+	+	2	2	3	3
	3974	F	1	1	+	1	2	2	3	3
	3975	F	0	0	+	+	1	+	2	2
									L R	L R
"Negative Control"	3961	M	0	0	0	0	0	0	0 0	0 0
(80% ethanol)	3962	M	0	0	0	0	0	0	0 0	0 0
	3963	M	0	0	0	0	0	0	0 0	0 0
	3964	M	0	0	0	0	0	0	0 0	0 0
	3965	M	0	0	0	0	0	0	0 0	0 0
	3966	F	0	0	0	0	0	0	0 0	0 0
	3967	F	0	0	0	0	0	0	0 0	0 0
	3968	F	0	0	0	0	0	0	0 0	0 0
	3969	F	0	0	0	0	0	0	0 0	0 0
	3970	F	0	0	0	0	0	0	0 0	0 0

L = Left flank (80% ethanol
R = Right flank (BDNPA/F-DPA)

Table VII. Evaluation of Five Unicharge Propellants in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Incidence and Severity of Responses at Challenge

PH 424-US-001-91

Study Number Treatment Group	Challenge Naive Site 24 hours Incidence	 Severity	 48 hours Incidence	 Severity
PH 424-US-001-91				
MeNENA	0/20	0.0	0/20	0.0
Negative Control-				
MeNENA	0/10	0.0	0/10	0.0
Distilled Water	0/10	0.0	0/10	0.0
Positive Control	5/5	2.6	5/5	2.0
PH 424-US-002-91				
EtNENA	0/20	0.0	0/20	0.0
Negative Control-				
EtNENA	0/10	0.0	0/10	0.0
80% EtOH	0/10	0.0	0/10	0.0
Positive Control	5/5	3.0	5/5	2.6
PH 424-US-003-91				
BuNENA	0/20	0.0	0/20	0.0
Negative Control-				
BuNENA	0/10	0.0	0/10	0.0
80% EtOH	0/10	0.0	0/10	0.0
Positive Control	5/5	2.8	5/5	2.8
PH 424-US-004-91				
BDNPA/F+DPA	0/19*	0.0	0/19*	0.0
Negative Control-				
BDNPA/F+DPA	0/10	0.0	0/10	0.0
80% EtOH	0/10	0.0	0/10	0.0
Positive Control	5/5	2.6	5/5	2.8
PH 424-US-005-91				
BDNPA/F-DPA	0/20	0.0	0/20	0.0
Negative Control-				
BDNPA/F-DPA	0/10	0.0	0/10	0.0
80% EtOH	0/10	0.0	0/10	0.0
Positive Control	5/5	2.6	5/5	2.4

*Animal euthanized, N=19

Table VIII. Evaluation of MeNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-001-91

Treatment Group Animal Number Test Group	Sex	Initial	Final
3801	M	357	583
3802	M	372	651
3803	M	393	670
3804	M	387	627
3805	M	373	619
3806	M	341	566
3807	M	353	560
3808	M	371	615
3809	M	361	628
3810	M	359	607
$\bar{x}$		366.7	612.6
S.D.		15.72	35.13
N		10	10
3811	F	370	602
3812	F	350	551
3813	F	341	498
3814	F	336	465
3815	F	343	503
3816	F	359	523
3817	F	323	486
3818	F	334	476
3819	F	359	556
3820	F	349	498
$\bar{x}$		346.4	515.8
S.D.		13.92	42.41
N		10	10

Table VIII. (cont'd) Evaluation of MeNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-001-91

Treatment Group Animal Number	Sex	Initial	Final
Negative Control			
3821	M	361	537
3822	M	348	567
3823	M	375	619
3824	M	364	600
3825	M	375	612
$\bar{x}$		364.6	587.0
S.D.		11.24	34.34
N		5	5
3826	F	352	557
3827	F	356	551
3828	F	339	531
3829	F	327	434
3830	F	338	529
$\bar{x}$		342.4	520.4
S.D.		11.68	49.82
N		5	5
Positive Control			
3831	M	364	530
3832	M	413	709
3833	M	348	525
$\bar{x}$		375.0	588.0
S.D.		33.87	104.82
N		3	3
3834	F	351	553
3835	F	347	508
$\bar{x}$		349.0	535.5
S.D.		2.83	31.82
N		2	2

Table IX. Evaluation of EtNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-002-91

Treatment Group Animal Number Test Group	Sex	Initial	Final
3836	M	394	691
3837	M	350	563
3838	M	366	597
3839	M	396	708
3840	M	378	693
3841	M	334	532
3842	M	343	548
3843	M	385	638
3844	M	361	561
3845	M	348	568
$\bar{x}$		365.5	609.9
S.D.		21.97	66.92
N		10	10
3846	F	354	543
3847	F	323	478
3848	F	345	500
3849	F	367	473
3850	F	326	594
3851	F	353	551
3852	F	362	513
3853	F	339	525
3854	F	328	470
3855	F	354	566
$\bar{x}$		345.1	521.3
S.D.		15.52	42.18
N		10	10

Table IX. (cont'd) Evaluation of EtNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-002-91

Treatment Group Animal Number	Sex	Initial	Final
Negative Control			
3856	M	376	637
3847	M	411	699
3858	M	371	607
3859	M	328	533
3860	M	383	624
$\bar{x}$		373.8	620.0
S.D.		29.91	59.76
N		5	5
3861	F	382	553
3862	F	318	513
3863	F	352	511
3864	F	363	545
3865	F	345	556
$\bar{x}$		352.0	535.6
S.D.		23.59	21.93
N		5	5
Positive Control			
3866	M	349	593
3867	M	346	536
$\bar{x}$		347.5	564.5
S.D.		2.12	40.31
N		2	2
3868	F	312	374
3869	F	347	556
3870	F	357	541
$\bar{x}$		338.7	490.3
S.D.		23.63	101.03
N		3	3

Table X. Evaluation of BuNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-003-91

Treatment Group Animal Number Test Group	Sex	Initial	Final
3871	M	350	554
3872	M	367	611
3873	M	354	647
3874	M	313	587
3875	M	347	570
3876	M	367	621
3877	M	361	608
3878	M	404	619
3879	M	356	574
3880	M	358	531
$\bar{x}$		357.7	592.2
S.D.		22.40	35.29
N		10	10
3881	F	341	503
3882	F	364	516
3883	F	334	475
3884	F	351	564
3885	F	320	422
3886	F	338	532
3887	F	305	448
3888	F	338	547
3889	F	323	476
3890	F	342	554
$\bar{x}$		335.6	503.7
S.D.		16.55	47.57
N		10	10

Table X. (cont'd) Evaluation of BuNENA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-003-91

Treatment Group Animal Number	Sex	Initial	Final
Negative Control			
3891	M	324	522
3892	M	328	498
3893	M	399	679
3894	M	335	553
3895	M	390	614
$\bar{x}$		355.2	573.2
S.D.		36.23	73.39
N		5	5
3896	F	314	535
3897	F	305	461
3898	F	343	528
3899	F	357	504
3900	F	330	527
$\bar{x}$		329.8	511.0
S.D.		21.09	30.29
N		5	5
Positive Control			
3901	M	406	606
3902	M	356	554
3903	M	351	527
$\bar{x}$		371.0	559.0
S.D.		30.41	41.58
N		3	3
3904	F	331	428
3905	F	343	549
$\bar{x}$		337.0	488.5
S.D.		8.49	85.56
N		2	2

Table XI. Evaluation of BDNPA/F+DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-004-91

Treatment Group Animal Number Test Group	Sex	Initial	Final
3906	M	367	573
3907	M	361	681
3908	M	398	584
3909	M	358	556
3910	M	386	607
3911	M	344	374*
3912	M	367	580
3913	M	375	629
3914	M	356	546
3915	M	330	518
$\bar{x}$		364.2	586.0
S.D.		19.56	48.28
N		9	9
3916	F	348	493
3917	F	360	569
3918	F	356	483
3919	F	367	566
3920	F	323	480
3921	F	321	487
3922	F	340	490
3923	F	348	511
3924	F	352	488
3825	F	351	495
$\bar{x}$		346.6	506.2
S.D.		14.88	33.38
N		10	10

* Animal euthanized, data not included in statistical analysis

Table XI. (cont'd) Evaluation of BDNPA/F+DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-004-91

Treatment Group Animal Number	Sex	Initial	Final
Negative Control			
3926	M	367	593
3927	M	369	577
3928	M	331	523
3929	M	382	580
3930	M	380	633
$\bar{x}$		365.8	581.2
S.D.		20.54	39.46
N		5	5
3931	F	358	502
3932	F	354	558
3933	F	376	601
3934	F	331	489
3935	F	381	468
$\bar{x}$		360.0	523.6
S.D.		19.86	54.61
N		5	5
Positive Control			
3936	M	384	627
3937	M	384	623
$\bar{x}$		384.0	625.0
S.D.		0.0	2.83
N		2	2
3938	F	347	497
3939	F	350	437
3940	F	345	463
$\bar{x}$		347.3	465.7
S.D.		2.52	30.09
N		3	3

Table XII. Evaluation of BDNPA/F-DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-005-91

Treatment Group Animal Number Test Group	Sex	Initial	Final
3941	M	346	590
3942	M	386	640
3943	M	386	589
3944	M	337	551
3945	M	369	553
3946	M	360	564
3947	M	406	693
3948	M	376	658
3949	M	385	577
3950	M	326	576
$\bar{x}$		367.7	599.1
S.D.		25.21	48.12
N		10	10
3951	F	335	503
3952	F	346	481
3953	F	310	459
3954	F	376	544
3955	F	348	507
3956	F	338	563
3957	F	330	493
3958	F	374	565
3959	F	322	517
3860	F	383	614
$\bar{x}$		346.2	524.6
S.D.		24.43	46.56
N		10	10

Table XII. (cont'd) Evaluation of BDNPA/F-DPA in the Delayed Contact Hypersensitivity Study in Guinea Pigs (Buehler Assay)

Summary of Body Weights (g)

PH 424-US-005-91

Treatment Group Animal Number	Sex	Initial	Final
Negative Control			
3961	M	384	650
3962	M	395	674
3963	M	352	622
3964	M	355	642
3965	M	373	611
$\bar{x}$		371.8	639.8
S.D.		18.46	24.62
N		5	5
3966	F	351	506
3967	F	353	546
3968	F	352	539
3969	F	394	636
3970	F	350	540
$\bar{x}$		360.0	553.4
S.D.		19.04	48.76
N		5	5
Positive Control			
3971	M	380	599
3972	M	386	642
3973	M	364	591
$\bar{x}$		376.7	610.7
S.D.		11.37	27.43
N		3	3
3974	F	346	471
3975	F	329	495
$\bar{x}$		337.5	483.0
S.D.		12.02	16.97
N		2	2

QUALITY ASSURANCE UNIT STATEMENT

Study Nos.: PH 424-US-001-91
PH 424-US-002-91
PH 424-US-003-91
PH 424-US-004-91
PH 424-US-005-91

Study Director: Susan E. Armondi, LAT

The Quality Assurance Unit conducted the inspections listed below and reported the results to the study director and to management on the dates indicated.

The following inspections were performed:

Interval	Date
In Life Phase	11/7/91, 11/7/91, 11/7/91 11/8/91, 11/8/91
Reporting Phase	1/24/92

Date QAU Report Issued

To Study Director	To Management
1/21/92	1/24/92

Leslie Pinnell
Quality Assurance

May 20, 1992
Date

COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practices (GLP) as promulgated by the following regulatory agencies, except that stability samples for archive retention were not maintained at the testing facility due to the nature of the compounds. Samples were returned to the sponsor and the retention of the samples will be the responsibility of the sponsor.

U.S. Food and Drug Administration as stated in 58 CFR 21.

Organization for Economic Co-operation and Development Guidelines for Testing Chemicals (OECD), ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12, 1981.

U.S. Environmental Protection Agency as stated in 40 CFR Parts 160 and 792.

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PH 424-US-002-91
PH 424-US-003-91
PH 424-US-004-91
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To the best of my knowledge, this study was conducted in accordance with applicable Good Laboratory Practice regulations; there were no deviations from these regulations that impacted on study conclusions.

Susan E. Richmond
Study Director

May 20, 1992
Date